channel function using planar lipid bilayers (PLBs) and integrity using analytical size exclusion chromatography (A-SEC) in the detergent-solubilized state. We also examined receptor mobility on the lipidic cubic phase (LCP) by measuring nAChR mobile fraction and diffusion coefficient through fluorescence recovery after photobleaching (FRAP) experiments using lipid-analog and non-lipid-analog detergents. Our results show that it is possible to isolate stable and functional nAChRs using lipid-analog detergents, with characteristic ion channel currents in PLBs and minimal aggregation as observed in A-SEC. Furthermore, fractional mobility and diffusion coefficient values observed in FRAP experiments were similar to the values observed for these parameters in the recently LCP-crystallized ß2-adrenergic receptor. The overall results show that phospholipid-analog detergents with 16 carbon acyl-chains support nAChR integrity, functionality, and LCP mobility.

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X-Ray Structures of General Anaesthetics Bound to a Pentameric Ligand-Gated Ion Channel

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General anaesthetics have enjoyed long and widespread use but their molecular mechanism of action remains poorly understood. There is good evidence that their principal targets are pentameric ligand-gated ion channels (pLGICs) such as inhibitory GABAA (γ-aminobutyric acid) receptors and excitatory nicotinic acetylcholine receptors, which are respectively potentiated and inhibited by general anaesthetics. The bacterial homologue from Gloeobacter violaceus (GLIC), whose X-ray structure was recently solved, is also sensitive to clinical concentrations of general anaesthetics. Here we describe the crystal structures of the complexes propofol/GLIC and desflurane/GLIC. These reveal a common general-anaesthetic binding site, which pre-exists in the apo-structure in the upper part of the transmembrane domain of each protomer. Both molecules establish van der Waals interactions with the protein; propofol binds at the entrance of the cavity whereas the smaller, more flexible, desflurane binds deeper inside. Mutations of some amino acids lining the binding site profoundly alter the ionic response of GLIC to protons, and affect its general-anaesthetic pharmacology. Molecular dynamics simulations, performed on the wild type and two GLIC mutants, highlight differences in mobility of propofol in its binding site and help to explain these effects. These data provide a novel structural framework for the design of general anaesthetics and of allosteric modulators of brain pLGICs.

Nury H, Van Renterghem C, Weng Y, Tran A, Dufresne V, Baaden M, Changeux JP, Sonner JM, Delarue M, Corringer PJ. Nature 469, 428-431 (2011).

599-Pos Board B385

New Estimates for the 'Allosteric' Constant of Neuromuscular Acetylcholine Receptor-Channels

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Nicotinic acetylcholine receptors (AChRs) open rarely in the absence of agonists but many different mutations substantially increase the unliganded gating equilibrium constant (the 'allosteric' constant, E_0). In the adult mouse neuromuscular AChR a single mutation, 'Special character' A96H (in both 'Special character' subunits), increased E_0 by $>10^5$-fold and caused spontaneous openings to occur in clusters arising from individual receptors. We measured E_0 for three different sets of mutant combinations and by extrapolation estimated E_0 for wild type AChRs. The estimates were E_0^{wt}=6.0x10^{-7} and 7.4 x 10^{-7} in adult-type AChRs and 4.3x10^{-7} in fetal-type AChRs (−100 mV, 23 °C). The adult value is in excellent agreement with one obtained previously by using a completely different method (6.6x10^{-7}, from monoliganded gating). We found that E_0^{wt} decreased e-fold with a ~58 mV depolarization, and that Na^+ and K^+ in the extracellular solution had no effect on E_0. However, Cs^+ acted as a very weak 'partial' agonist. The results are discussed with regard to the energetics of receptor activation and the competitive antagonism of ions with regard to agonist binding.

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Identification of Amino Acid Residues that Prevent Efficient Binding of 4/7 α-Conotoxins to Nicotinic α4β2 Receptor Subtypes

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α-Conotoxins are nAChR antagonists with high subtype selectivity. Several potent α3β2 nAChR-selective α-contoxins have been identified. However, most characterized α-conotoxins show no or only weak affinity for the α4β2 nAChR subtype. In this study, we constructed homology models of α3β2 and α4β2 ligand binding domains and substituted selected amino acid residues in the α4 subunit by the respective residues in the α3 subunit to identify the determinants of subtype selectivity. Characterization of these mutants by two-electrode voltage-clamp analysis identified two mutants, α4R185I and α4P195Q, that exhibited increased affinity for several α-conotoxins. Replacement of α4R185 by alanine or glutamate but not by lysine also increased α-conotoxin affinity, indicating that a positive charge in this position interferes with α-conotoxin binding. Docking studies and molecular dynamic simulations supported our findings and further demonstrated that residues R185 and P195 inhibit a close contact of the C-loop with α-conotoxins and thus prevent high affinity binding.

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On the Complexity of the Kinetics of Unliganded Gating in Neuromuscular Acetylcholine Receptor-Channels

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The magnitude of an agonist-induced response depends on both the intrinsic tendency of the unliganded receptor to open and the amount of agonist binding energy realized in the channel-opening process. It is easy to make mouse neuromuscular acetylcholine receptors (AChRs) open in the absence of agonist by making a small number of mutations (cell attached, HEK cells, −100 mV). However, the single-channel, unliganded gating kinetics for an individual AChR is complex and show multiple closed and open components (3C, 2O). We have found that many perturbations (in the vicinity of the transmitter binding site) increase or decrease this complexity. Mutations at positions αA96, αG153, αF189, or εP121(δP123) increase the probability of longer openings, as does the presence of extracellular Cs+. Agonists and most mutations at positions αY93, αG147, αW149, αY190 or αY198 greatly reduce these events. We compared log-likelihood values for all possible 3C-2O kinetic schemes (without cycles) and found that four of these consistently produced equivalently-high scores. However, only some were able to reproduce the rapid rising phase of the synaptic response apparent under normal physiological conditions. Although we as yet do not have an understanding of the structural basis for the kinetic complexity, the results suggest that without ligands there is a conformational flexibility at the binding site that is absent with ligands. The malleability of unliganded gating kinetics further suggests that many binding site residues can influence this flexibility. Supported by NS-23513 and NS-64969.

602-Pos Board B388

Effects of Mutations of Proline Residues Located in the Complementary Subunit of the Acetylcholine Receptor Transmitter Binding Site

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Nicotinic acetylcholine receptor-channels (AChRs) are allosteric proteins that isomerize between a non-conducting/low-affinity and a conducting/high-affinity conformation. Each neuromuscular AChR has two transmitter binding sites located at the interface between adjacent subunits ($\alpha_\varepsilon$or α_δ.We studied the gating properties of AChRs with mutations at the vicinal prolines located at these interfaces. In both cases, mutations of the N-terminal residue had little effect but those of the C-terminal residue (εP121 or δP123) decreased substantially the open channel probability (500 μM ACh). We estimated the unliganded (E_0) and diliganded (E_2) gating equilibrium constants for mutations of these two prolines from single-channel currents (30 mM ACh, +100mV). All of the tested substitutions decreased both gating constants. The slope of the rate-equilibrium plot (Φ)for εP121/δP123 was ~0.96 (diliganded) and ~0.70 (unliganded). Some of the mutations caused a large decrease in the closed/open equilibrium dissociation constant ratio for ACh (λ^{ACh}) while others had more modest effects. Probing the effects of εP121 and δP123 mutations should allow an estimation of the binding and gating properties of each of the two transmitter binding sites, separately.

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Introducing Kappa: An Integrated, 'Catch-And-Hold' Reaction Co-Ordinate that Triggers AChR Gating

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For many years drug action on membrane receptors has been conceptualized as occurring in two distinct steps, initially termed 'affinity' and 'intrinsic activity'. In ligand-gated ion channels these stages of activation are called 'binding' and 'gating'. Single-channel kinetic analysis of adult-type mouse neuromuscular